PB84-112325

Toxicity of Silver to Steelhead and Rainbow Trout, Fathead Minnows and Daphnia Magna

Corvallis Environmental Research Lab., OR

1983

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1. REPORT NO. EPA-600/J-83-077	2.
3. PB84-112325	
4. TITLE AND SUBTITLE: Toxicity of Silver to Steelhead and Rainbow Trout, Fathead Minnows and Daphnia Magna (Journal Version)	5. REPORT DATE: 1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Alan V. Nebeker, Chris K. McAuliffe, Roger Mshar, and Donald G. Stevens	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Research Laboratory, Office of Research and Development, USEPA, Corvallis, OR 97333	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: same	13. TYPE OF REPORT AND PERIOD COVERED: journal article
	14. SPONSORING AGENCY CODE: EPA/600/02

15. SUPPLEMENTARY NOTES
Environmental Toxicology and Chemistry 2: 95-104, 1983

16. ABSTRACT

Rainbow (Salmo gairdneri) and steelhead (Salmo gairdneri) trout and fathead minnows (Pimephales promelas) were exposed to silver for 96 h in replicated flow-through tests. Rainbow trout, fathead minnows and Daphnia magna were exposed to silver in replicated static acute tests. Steelhead trout embryo-larval stages were tested in a flow-through system for 60 d, and D. magna were exposed through complete life cycles in replicated static-renewal tests. All silver concentrations were measured values. No differences were seen between flow-through and static LC_{50} values. Rainbow trout 96-h flow-through LC_{50} values were 8.6 and 9.7 µg/L silver; static values were 10.9 and 8.5 µg/L. The 96-h LC_{50} for steelhead trout in the flow-through test was 9.2 µg/L. Fathead minnow 96-h flow-through LC_{50} values were 5.6 and 7.4 µg/L silver; static values were 9.4 and 9.7 µg/L. The absence of food in static tests with D. magna caused silver to be about 10 times more toxic; the 48-h static mean EC_{50} value for D. magna without food was 0.9 µg/L, compared to 12.5 µg/L in the test with food. The early-life-stage test with steelhead trout, from newly fertilized eggs to post-swimup juveniles, showed complete mortality at 1.3 µg/L and significant reduction in fish survival at 0.5 µg/L silver. Mean weight and length at end of test were greatly reduced at 1.1 µg/L, and were significantly different from controls at 0.1 µg/L. The mean 21-d EC_{50} value for the D. magna test with food was 3.5 µg/L. The 21-d lowest significant ($\alpha = 0.05$) effect on survival occurred at 4.1 µg/L.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: Release to public	19. SECURITY CLASS *(This Report)*: unclassified	21. NO. OF PAGES: 10
	20. SECURITY CLASS *(This page)*: unclassified	22. PRICE

EPA Form 2220-1 (9-73)

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SPRINGFIELD, VA. 22161

EPA-600/J-83-077
JOURNAL ARTICLE

Environmental Toxicology and Chemistry, Vol. 2, pp. 95-104, 1983
Printed in the USA. Pergamon Press Ltd.

0730-7268/83/010095-10$03.00/0
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TOXICITY OF SILVER TO STEELHEAD AND RAINBOW TROUT, FATHEAD MINNOWS AND *DAPHNIA MAGNA*

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(*Received* 20 *September* 1982; *Accepted* 8 *December* 1982)

Abstract — Rainbow (*Salmo gairdneri*) and steelhead (*Salmo gairdneri*) trout and fathead minnows (*Pimephales promelas*) were exposed to silver for 96 h in replicated flow-through tests. Rainbow trout, fathead minnows and *Daphnia magna* were exposed to silver in replicated static acute tests. Steelhead trout embryo-larval stages were tested in a flow-through system for 60 d, and *D. magna* were exposed through complete life cycles in replicated static-renewal tests. All silver concentrations were measured values. No differences were seen between flow-through and static LC_{50} values. Rainbow trout 96-h flow-through LC_{50} values were 8.6 and 9.7 μg/L silver; static values were 10.9 and 8.5 μg/L. The 96-h LC_{50} for steelhead trout in the flow-through test was 9.2 μg/L. Fathead minnow 96-h flow-through LC_{50} values were 5.6 and 7.4 μg/L silver; static values were 9.4 and 9.7 μg/L. The absence of food in static tests with *D. magna* caused silver to be about 10 times more toxic; the 48-h static mean EC_{50} value for *D. magna* without food was 0.9 μg/L, compared to 12.5 μg/L in the test with food. The early-life-stage test with steelhead trout, from newly fertilized eggs to post-swimup juveniles, showed complete mortality at 1.3 μg/L and significant reduction in fish survival at 0.5 μg/L silver. Mean weight and length at end of test were greatly reduced at 1.1 μg/L, and were significantly different from controls at 0.1 μg/L. The mean 21-d EC_{50} value for the *D. magna* test with food was 3.5 μg/L. The 21-d lowest significant ($\alpha = 0.05$) effect on survival occurred at 4.1 μg/L. The mean silver concentration at which there was a significant decrease in total *Daphnia* young/female/day after 21 d, compared to the control was 10.5 μg/L silver.

Keywords — Silver Steelhead trout Rainbow trout Fathead minnow *Daphnia magna* Toxicity

INTRODUCTION

This paper presents information on the comparative acute and chronic sensitivity to silver ($AgNO_3$) of four freshwater animals: rainbow trout (*Salmo gairdneri*), steelhead trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*) and *Daphnia magna*. The purpose of this study was to (a) compare acute effects of silver on four organisms, (b) compare static and flow-through acute fish test results, (c) compare acute and chronic test results and relationships for steelhead trout and *D. magna*, and (d) to investigate the ability of acute tests to predict chronic effects.

There is relatively little information in the literature concerning the toxicity of silver to freshwater organisms [1]. It is one of the most toxic of the heavy metals, but there are no large industrial losses because of its value as a recoverable waste. However, mining and ore processing discharges and drainage from old mines and tailings can be a problem [2]. Acute toxicity silver values for fish (96-h LC_{50}) range from 3.9 μg/L [3] for fathead minnows in soft water to 280 μg/L for rainbow trout in hard water (Lemke, A.E., unpublished). Hale [4] obtained a 96-h LC_{50} value of 28.8 μg/L in a

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flow-through measured test with rainbow trout. Davies et al. [5] obtained a 96-h LC_{50} of 5.3 μg/L for rainbow trout in soft water. No embryo-larval tests for silver toxicity have been conducted previously with steelhead trout, but three tests have been completed with rainbow trout: one using silver nitrate by Davies et al. [5], starting with eyed eggs, and two with silver iodide [6], one starting with eyed eggs and one starting with newly fertilized (green) eggs. The chronic studies by Davies [2] with rainbow trout using silver showed long-term mortality at concentrations as low as 0.13 μg/L. Nehring [7] found that the mayfly *Ephemerella grandis* was more sensitive than were rainbow trout, with a 14-d LC_{50} of < 1.0 μg/L silver; the stonefly *Pteronarcys californica* had a 14-d LC_{50} of 4.0–9.0 μg/L Silver. Anderson [8], using silver nitrate in Lake Erie water, showed that *D. magna* did not survive at silver concentrations >5.1 μg/L. Bringmann and Kühn [9] reported a 24-h LC_{50} of 1.5 μg/L silver. The U.S. Environmental Protection Agency (EPA) [10] presented a static unmeasured 48-h LC_{50} test value of 1.5 μg/L for *D. magna*. Additional information on the toxicity of silver is needed to adequately protect aquatic life.

METHODS AND MATERIALS

Water

Unchlorinated well water was used for all testing, and was aerated vigorously to saturation before use. Water temperature and hardness during acute testing with fish are listed in Table 1. Dissolved oxygen was maintained near saturation for all fish tests. Total hardness (as $CaCO_3$), alkalinity (as $CaCO_3$) and pH during the steelhead trout tests were 36 mg/L, 25 mg/L and 7.0 respectively. Dissolved chloride concentrations in our water typically range from 3.0 to 21.0 mg/L, with a mean of 7.2 mg/L [11]. Water hardness for the two *Daphnia* acute tests was 38–40 mg/L; pH was 7.5 and alkalinity was 31 mg/L. Water hardness, alkalinity and pH during the two *Daphnia* acute tests with and without food were 33 mg/L, 27 mg/L and 7.2, respectively. Water hardness

Table 1. Acute silver toxicity to *Daphnia magna*, steelhead and rainbow trout and fathead minnow

Species	Test[a] type	96-h[b] LC_{50} (μg/L)	Confidence limits (95%)	Mean fish fork length (mm)	Mean fish wt (g)	Mean temp (°C)	Mean water hardness (mg/L)
Daphnia	Static	1.1[d]	0.8– 1.5	(no food)	—	20	38
Daphnia	Static	0.6[d]	0.5– 0.8	(no food)	—	20	40
Daphnia	Static	1.1[d]	1.0– 1.1	(no food)	—	20	33
Daphnia	Static	12.5[d]	10.9– 14.3	(with food)	—	20	33
Steelhead	Flow-through	9.2	—	32	0.2	12	36
Rainbow	Static	72.9	60 – 88	78	6.6	10	40
Rainbow	Static	84.4	71 –100	78	6.6	9	37
Rainbow	Static[c]	10.9	9.2– 13.0	28	0.2	12	26
Rainbow	Static[c]	8.5	7.1– 10.2	44	0.3	12	35
Rainbow	Flow-through	8.6	8.0– 9.2	28	0.2	12	29
Rainbow	Flow-through	9.7	8.4– 11.3	34	0.4	12	42
Fathead	Static	9.4	8.4– 10.4	26	0.3	20	38
Fathead	Static	9.7	8.5– 11.2	20	0.1	21	39
Fathead	Flow-through	5.6	4.5– 6.4	24	0.1	22	40
Fathead	Flow-through	7.4	7.2– 11.1	26	0.2	22	36

[a] Daphnia tests unaerated; fish static tests aerated (except those designated by "c").
[b] From measured concentrations.
[c] Tests unaerated.
[d] 48-h EC_{50}.

during *Daphnia* chronic test no. 1 was 60 mg/L (as $CaCO_3$); pH was 7.2 and total alkalinity was 49 mg/L (as $CaCO_3$). Water to be used for one complete test was stored in a 400-liter polyethylene tank. For *Daphnia* chronic tests nos. 2 and 3, to compare the impact of water hardness on *Daphnia* survival and reproduction, additional chemicals (as specified in the American Society for Testing and Materials (ASTM) method [12] for preparing reconstituted water) were added to increase the hardness of our well water from 60 mg/L (test no. 1) to 75 mg/L (test no. 2) and 180 mg/L (test no. 3). Routine water chemistries were obtained several times during each test using standard methods [13].

Toxicant

Reagent grade silver nitrate ($AgNO_3$) was used for all testing except in the *Daphnia* fed and unfed tests, in which silver wire was dissolved in nitric acid and used to prepare stock solutions. *Daphnia* 48-h EC_{50} bioassay results with silver nitrate and silver wire were identical (1.1 μg/L), indicating that both methods produce the same silver form. A Perkin-Elmer 305b atomic absorption spectrophotometer, equipped with a hollow graphite analyzer, was used to analyze for silver using standardized methods [14]. One stock solution, with standards, was prepared for each test. For the static *Daphnia* tests, solutions were prepared in sufficient volume to fill all test containers. Samples for chemical analyses were taken from the static-test containers at the beginning of the acute tests, and before and after renewing test concentrations during the *Daphnia* life-cycle tests. Spiked samples, blanks, etc., were routinely maintained for quality assurance. For example, the limit of detection for the fed-*Daphnia* test was 0.5 μg/L, with 99% spike recovery. The test concentration of the life-cycle tests was the arithmetic mean of all measured concentrations. Silver and food were added to the water and mixed thoroughly before the *Daphnia* were added. For the static fish tests, the silver was added to water in the test containers and mixed thoroughly before animals were added. Samples for chemical analysis of the static tests were taken from the test containers at the beginning of the tests. For the flow-through tests with rainbow and steelhead trout and fathead minnows, a pump delivered a stock silver solution to the diluters at a constant, predetermined rate, and average concentrations were determined from daily samples.

Fish acute tests

Rainbow trout from freshwater stream populations were obtained as eggs from the Oregon Department of Fish and Wildlife Oak Ridge Hatchery, and were hatched and reared at Western Fish Toxicology Station (WFTS), Corvallis, Oregon. Steelhead trout juveniles from sea-run populations were taken from extra control fish reared from the early-life-stage test. The fathead minnows were hatched and reared from WFTS cultures; the original fish were collected in 1978 from Oregon Department of Fish and Wildlife ponds near Aumsville, Oregon. Fish were not fed during acute testing.

The methods used for the 96-h flow-through and static acute tests with rainbow and steelhead trout and fathead minnows were basically those presented in the *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians* [12]. Five silver concentrations plus a control were used for all tests, with a 50% dilution ratio between concentrations. Static rainbow trout and fathead minnow tests were conducted using aerated or unaerated 20-liter jars, depending on fish size. Ten fish were placed in each jar, with two jars per concentration. Flow-through tests with rainbow trout, fathead minnows, and steelhead trout were conducted in 20-liter aquaria, using a Garton diluter water delivery system [15], with 520 ml/min delivered to each aquarium. Ten fish were randomly assigned to each aquarium, with two

aquaria per concentration; dead fish were counted and removed daily.

Steelhead trout early-life-stage test

Eggs for this test were obtained from sea-run steelhead trout populations at the Oregon Department of Fish and Wildlife North Fork (Alsea River) hatchery (~7°C). Eggs and sperm were placed together in pint jars at the hatchery without water and transported to the WFTS laboratory in an insulated box at 9°C. Transit time was about 30 min. Fertilization was initiated by placing the egg and sperm mixture in incubator trays filled with 9°C water. There was no water change for the first 2 min; water was then delivered to and flowed through the tray at ~20 l/min for 1 h. The fertilized eggs for the early-life-stage test were then transferred to the test chambers.

The experimental method for fertilizing eggs used for the steelhead trout embryo-larval test, i.e., transferring eggs and sperm together and then adding water about an hour later, resulted in only ~50% fertilization, and we do not recommend it. For the rainbow trout used in this study, and for most other fish at WFTS, we fertilized eggs at the hatchery immediately after stripping the adults, waited 1 h for the eggs to water-harden, and then transported the eggs to the laboratory; this normally results in >95% fertilization.

A diluter producing 10 silver concentrations, using a toxicant delivery system similar to that of Garton [15], was used for the early-life-stage test. Oval stainless steel troughs, 10 cm wide with a water depth of 7 cm, were the test chambers for eggs and young fish. To hold the eggs in place, wooden dowels, 1.5 cm in diameter and 10 cm long, were placed lengthwise and glued at 7-cm intervals to the bottom of the test chambers, perpendicular to the water flow. Equal volumes (eggs were counted later) of newly fertilized eggs (~100/vol) were placed in the compartments between the dowels. Water flow, maintained by jetting the incoming water from the diluter into the oval troughs, was sufficient to circulate water across the eggs but not great enough to disturb or roll them. Low light intensity was maintained on a natural photoperiod. Water temperature was 9.0°C on the first day and then gradually was raised to 12°C (0.5 degrees per day) for the remainder of the 60-d test.

Embryos and fish were exposed to silver from 1 h post-fertilization through to the post-swimup juvenile developmental stage. Eggs were considered dead when they turned white; they were counted and removed daily. Fish were fed newly hatched brine shrimp several times daily when feeding became necessary. Time to swimup was recorded, and mortality was calculated during and at the end of the test. Weight and fork-length of fish at the end of the test were also recorded.

Daphnia *acute and life-cycle tests*

D. magna are maintained in culture at our WFTS laboratory. They were acclimated to test conditions prior to silver exposure and were given the same food during rearing and chronic testing. Original cultures were obtained from the National Water Quality Laboratory (EPA), Duluth, Minnesota, in 1971. *Daphnia* cultures were fed blended trout chow and yeast (12 g PR-11 trout chow plus 3 g yeast/L water) at a level of ~10 mg solids/L test water. Cultures for the fed and unfed acute tests were also given 2 mg/L of the algae *Selenastrum capricornutum*. During the life-cycle tests and the fed and unfed acute tests, they were given 30 mg/L, as recommended by ASTM [16], but they were not fed during the two acute tests.

The methods used for the acute tests with *D. magna* are those presented in the *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians* [12]. All tests were conducted in a room held at a constant temperature of 19.5 ± 1°C. Light was set at

16 h daylight and 8 h darkness. Lights were wide-spectrum Grolux and cool-white fluorescent bulbs set at 25 to 30 ft-c at bench-top. Two preliminary acute tests without food were conducted, with five concentrations — 4.0, 2.0, 1.0, 0.5 and 0.25 μg/L nominal — plus a control for each test, using a 50% dilution ratio between concentrations. Beakers were covered with glass plates to prevent evaporation of test water and contamination of the test solutions. *Daphnia* were tested in 200 ml water contained in 250-ml beakers. Five daphnids < 24-h old were placed in each beaker, with four replicates at each concentration.

Two additional acute tests were conducted, one without food and one with food. The food was PR-11 plus yeast fed at a concentration of 30 mg/L solids. Two beakers, each with 10 animals in 200 ml water, were used at each concentration. Seven concentrations plus controls were used for each of the two tests, ranging from a high of 40 μg/L silver in the fed test to a low of 0.15 μg/L silver in the unfed test.

The three *Daphnia* life-cycle tests with silver were conducted using modifications of Drafts 2 [17] and 4 [18] of the ASTM method for static-renewal life-cycle testing of *D. magna*. Ten 250-ml beakers (200 ml water) at each concentration, with five concentrations plus controls, were used for each test. One daphnid, < 24-h old, was placed in each beaker. The test temperature was also 19.5 ± 1°C, with a 16-h light:8-h dark photoperiod. Test solutions were renewed three times a week. The parent *Daphnia* was transferred to the fresh solution with a glass pipette (5 mm i.d.), and the young animals were counted and discarded at each renewal. The effects of silver on survival, total number of young produced and young/female/day were examined for 21 days. Average young/female/day is defined as the total number of young produced divided by the number of days that the female was producing young, i.e., time from the first day young were found to the end of the test or death of the parent.

Statistical analyses

The 96-h LC_{50} (lethal concentration, 50% mortality after 96 h) values were calculated for the fish using logistic regression methods [18]. One rainbow trout, one fathead minnow and the fed and unfed acute *Daphnia* tests were analyzed using the Trimmed Spearman-Karber method [19].

The survival data for the steelhead trout embryo-larval test were analyzed to determine significant differences from controls. Treatment effects were determined by first analyzing the survival data with a chi-square test for homogeneity. The data were then analyzed using the Williams multiple comparison test [20] to determine treatment levels that were significantly ($\alpha = 0.05$) different from the controls. The growth data were analyzed using Duncan's New Multiple Range Test. The use of the word significant throughout this paper denotes statistical significance tested at the 0.05 level.

Four 48-h EC_{50} values (effect concentration, 50% immobilization) from the four acute tests and three 21-d EC_{50} values from the three chronic tests were obtained for *Daphnia*. The EC_{50} values were determined using logistic regression methods [18]. Chi-square analyses were used to determine significant mortality of parents at 21 d, compared to controls. Reproductive data from each of the chronic tests were analyzed using analysis of variance and Dunnett's multiple comparison test [21] to obtain the maximum acceptable toxicant concentrations (MATC) based on reproduction. Geometric means of the MATC limits, as used by the U.S. EPA [1], were calculated for each of the three chronic tests.

RESULTS

Fish acute tests

Rainbow trout were sensitive to silver nitrate, with 96-h LC_{50} values as low as 8.5 μg/L (Table 1). Significant differences in two

pairs of rainbow trout silver static LC_{50} values — 72.9 and 84.4, and 10.9 and 8.5 μg/L — were probably caused by use of different-size fish in same-size 20-liter jars. The larger fish, with greater biomass, mucus, wastes, etc., caused more rapid binding and inactivation of the silver than did the smaller fish. Because there was less active silver available in the jars containing the larger fish, the LC_{50} values were higher. The somewhat lower temperatures at which the larger fish were tested probably did not affect the apparent toxicity. The 96-h LC_{50} values from the flow-through tests for rainbow trout (8.6 and 9.7 μg/L) and steelhead trout (9.2 μg/L) were not significantly different from each other; the two distinct races of *S. gairdneri* appear to have identical responses to acute silver exposure. Flow-through 96-h LC_{50} values for rainbow trout were similar to the static test values with the smaller fish. The fathead minnow 96-h LC_{50} values for silver, ranging from 5.6 to 9.4 μg/L, were similar to those for rainbow and steelhead trout. All four tests, static and flow-through, had very similar results (Table 1), with the flow-through test values being lower than the static LC_{50} values by a factor of 0.7.

Steelhead trout early-life-stage test

In the steelhead trout early-life-stage test, the exposure concentrations ranged from 1.95 to 0.10 μg/L silver. Complete mortality occurred at 1.3 μg/L and there was significant ($\alpha = 0.05$) reduction in fish survival, compared to the controls, at concentrations as low as 0.5 μg/L silver (Table 2). Mean weight and length of the fish at the end of the test were reduced at 1.1 μg/L, and were significantly different from the controls at 0.1 μg/L (Table 3). The estimated MATC, based on the lowest significant effect level, is < 0.1 μg/L silver.

Daphnia *acute and life-cycle tests*

The *Daphnia* were more sensitive to silver than were rainbow trout or fathead minnows in tests without food (Table 1). A mean EC_{50} of 0.9 μg/L was calculated from the three 48-h EC_{50} values, which were 1.1 (confidence interval (CI) 0.8–1.5), 1.1 (CI 1.0–1.12) and 0.6 (CI 0.5–0.8) μg/L silver. The CI were narrow, the EC_{50} values were similar and there was little control mortality. The 48-h EC_{50} with food was 12.5 (CI 10.9–14.3) μg/L, higher than the EC_{50} values determined without food (Table 1).

Tests conducted to characterize the chemistry of silver in glass beakers at 4 μg/L silver with the addition of food, as well as different methods of analyzing the samples, showed that the concentration of silver in

Table 2. Effect of silver nitrate on survival of steelhead trout in an early-life stage test

Mean measured silver conc. (μg/L ± SD)	Total number of eggs	Total larval hatch[a]		Survival of hatched fish at end of test	
		n	%	n	%
Control	233	123	53	100	83
1.95 ± 0.44	108	33	35	2	5[b]
1.32 ± 0.26	103	33	32	0	0[b]
1.06 ± 0.47	131	61	47	22	36[b]
0.70 ± 0.19	116	44	38	23	52[b]
0.51 ± 0.14	116	36	31	22	61[b]
0.36 ± 0.12	119	39	33	26	67
0.24 ± 0.08	124	46	37	41	89
0.20 ± 0.12	95	52	55	47	90
0.13 ± 0.06	105	62	59	51	82
0.10 ± 0.03	103	77	75	60	78

[a]Low percent hatch due to poor fertilization.
[b]Significantly different from controls (0.05) using Williams multiple comparison test.

Table 3. Effect of silver nitrate on the final weight and length of steelhead trout in an early life-stage test; means connected by the same line are not significantly different at the 0.05 level, using Duncan's New Multiple Range Test

	Silver concentration (μg/L)							
	Control	0.20	0.24	0.10	0.13	0.51	0.36	0.70	1.06
Mean fish weight at end of test (g)	31.7	30.0	29.8	29.5	29.4	28.9	28.6	28.1	24.7

	Silver concentration (μg/L)							
	Control	0.24	0.20	0.51	0.10	0.36	0.13	0.70	1.06
Mean fish length at end of test (mm)	0.26	0.24	0.24	0.235	0.23	0.22	0.22	0.21	0.16

unfiltered test water was close to nominal ($\bar{x}$ = 4.2 μg/L) in both distilled and well water. When well water plus silver was filtered (0.45 μm), 59% of the silver was lost; when food was added but not filtered, 42% of the silver was lost; when food was added then filtered, 89% of the silver was lost.

The first life-cycle test, with exposure concentrations ranging from 17.6 to 0.8 μg/L, showed effects on parent survival as well as significant reproduction effects on the total number of young and young/female/day at 4.1 μg/L silver and all higher concentrations (Table 4). The young/female/day, from the day of the first young until adult death or the end of the test, ranged from 4.1 animals at 17.6 μg/L silver to 13.4 animals in the controls. The total number of young ranged from 31 animals at 17.6 μg/L silver to 1,351 animals in the controls. The 21-d EC_{50} and MATC were 2.9 and 1.6 μg/L silver, respectively.

The second life-cycle test, with exposure concentrations ranging from 19.4 to 1.0 μg/L, showed a significant (α = 0.05) effect on parent survival, total young and young/female/day at 19.4 μg/L silver (Table 4). Reproduction at concentrations < 19.4 μg/L was not significantly lower than in the controls. Overall, reproduction in the second test was higher than in the first test, and some mortality occurred in the controls. The 21-d EC_{50} and the MATC were 3.6 and 8.8 μg/L silver, respectively.

The third life-cycle test, with exposure concentrations ranging from 30.8 to 3.4 μg/L, showed a significant (α = 0.05) effect on parent survival, total young and young/female/day at 8.0 μg/L silver and all higher levels. Greater mortality in the controls, apparently due to handling, reduced the number of young produced, compared to the first two tests. The 21-d EC_{50} and the MATC were 3.9 and 3.4 μg/L silver, respectively.

The results of the three *Daphnia* life-cycle tests indicate that water hardness did not affect the survival or reproduction of *D. magna* during chronic silver exposure. Three water hardness levels were tested — 60, 75 and 180 mg/L — which correspond to 21-d EC_{50} values of 2.9, 3.6 and 3.9 μg/L, respectively, and to 21-d geometric means of MATC values of 2.6, 13.1 and 5.2 μg/L, respectively (Table 5).

DISCUSSION

There was a large difference between the acute and chronic toxicity of silver to steelhead trout, as shown by a comparison of the 96-h LC_{50} and the toxic effect levels determined from the early-life-stage test. The 96-h LC_{50} was 9.2 μg/L, compared to a significant (α = 0.05) reduction in survival

Table 4. *Daphnia magna* life-cycle toxicity with silver nitrate

	Mean measured silver conc. (μg/L)	Adults alive and active	Total young	Mean young per adult/day
Test 1	17.6	0[a]	31[a]	4.1[a]
	7.6	0[a]	134[a]	5.5[a]
	4.1	6[a]	665[a]	8.7[a]
	1.6	9	1,297	13.7
	0.8	7	812	12.1
	Control	10	1,351	13.4
Test 2	19.4	0[a]	0[a]	0.0[a]
	8.8	7	934	14.0
	4.0	6	921	14.1
	1.8	5	1,122	17.0
	1.0	6	914	15.8
	Control	8	1,469	19.7
Test 3	30.8	0[a]	0[a]	0.0[a]
	24.5	0[a]	0[a]	0.0[a]
	20.1	0[a]	0[a]	0.0[a]
	8.0	1[a]	62[a]	4.4[a]
	3.4	6	503	7.1
	Control	7	680	8.9

[a]Significantly different from controls ($p \leq 0.05$).

at 1.1 μg/L and a significant ($\alpha = 0.05$) reduction in growth at 0.1 μg/L in the early-life-stage test. There was a positive relationship between silver exposure concentration and survival of fish, mean weight and length of fish at the end of the early-life-stage test. The acute-to-chronic application factor is calculated to be 0.01. The data reported by Davies et al. [5] agree with and support the conclusions of the present study. They reported that rainbow trout were affected by silver nitrate at 0.17 μg/L during an early-life-stage test, which started with eyed eggs, with a 96-h LC_{50} of 6.5 μg/L in the same water.

The LC_{50} values obtained with smaller rainbow trout in the static tests were similar to the rainbow trout flow-through test LC_{50} values, in which the silver is continuously replenished, indicating that the static LC_{50} values with the small fish accurately reflect the concentration of unbound silver available for uptake by the fish. It is known that silver and most other heavy metals actively bind with a variety of dissolved and suspended materials in water [14], which can

Table 5. Chronic toxicity of silver to *Daphnia magna* in static-renewal 21-d life-cycle tests conducted at 20°C and 15-h daylight

Chronic test no.	21-d EC_{50} (μg/L)	MATC[a] (μg/L)	Geometric mean of MATC values (μg/l)	Mean water hardness (mg/L as $CaCO_3$)
1	2.9	1.6[b] – 4.1[c]	2.6	60
2	3.6	8.8 – 19.4	13.1	75
3	3.9	3.4 – 8.0	5.2	180

[a]Maximum acceptable toxicant concentration based on survival and reproduction.
[b]No significant difference ($p = 0.05$) from controls.
[c]Significantly different ($p = 0.05$) from controls.

limit the available supply of free silver for uptake by the fish. We believe the presence of slime, fecal material, etc., in the test containers of the larger trout limited the availability of silver, and resulted in higher LC_{50} values, compared to the smaller fish and the flow through LC_{50} values.

The results of the present study show that the 21-d *Daphnia* EC_{50} values and the concentrations causing significant mortality ($\alpha = 0.05$) were as low or lower than the reproductive values, indicating that, for some chemicals, a simpler and less expensive test using just long-term survival might be adequate to predict toxicity. Either value, mortality or reproduction, could be used to determine the impact of silver on the life cycle. However, more research is needed to support the use of a simpler test.

It is also debatable, from the data obtained in this study, if short-term acute tests can predict the results of long-term exposure, or what may occur in the field, mainly due to the impact of complexing material, e.g., food in the water. The 48-h EC_{50} value obtained with food was higher than the 21-d EC_{50} with food and the acute values obtained without food, indicating that silver became unavailable to the *Daphnia* by the addition of food. The acute-to-chronic application factor was 0.27, using the mean 48-h EC_{50} without food and the mean geometric mean of MATC values. Nebeker [22] showed that food concentration also influenced the survival of *Daphnia* in chronic tests by decreasing available oxygen, in addition to decreasing the available or dissolved silver. Dissolved oxygen in the present study dropped to as low as 5.6 mg/L in the fed acute tests. Biesinger and Christensen [23] and Winner [24] also showed that the addition of organic matter as food altered the toxicity of heavy metals.

Acknowledgement — We wish to thank J. D. Andros for statistical analysis of the data; J. K. McCrady, A. Sipols, K. Wickman, W. L. Griffis and C. M. Wise for silver preparation and analyses; F. Recht and S. Ota for assistance with *Daphnia* testing; and the Oregon Department of Fish and Wildlife for steelhead and rainbow trout eggs. We also want to thank the journal reviewers for the extra effort given in review of this manuscript.

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